Sains Malaysiana 43(1)(2014): 89–94

Production of Major Mycotoxins by *Fusarium* Species Isolated from Wild Grasses in Peninsular Malaysia

(Penghasilan Mikotoksin Utama oleh Spesies *Fusarium* yang Dipencilkan daripada Rumput Liar di Semenanjung Malaysia)

I. NOR AZLIZA, R. HAFIZI, M. NURHAZRATI & B. SALLEH*

ABSTRACT

The Fusarium *species are notoriously known for causing various plants and animal diseases and producing a number of harmful mycotoxins. The mycotoxins production by species recovered from non-agricultural hosts such as wild grasses have hitherto never been given attention. We examined 30 strains representing 12* Fusarium *species i.e.* F. oxysporum, F. solani, F. semitectum, F. nelsonii, F. compactum, F. equiseti, F. chlamydosporum, F. proliferatum, F. subglutinans, F. sacchari, F. lateritium *and* F. incarnatum-equiseti *species complex isolated from wild grasses in Peninsular Malaysia for the production of four major mycotoxins i.e. moniliformin (MON), fumonisin B_1 (FB_1), zearalenone (ZEN) and beauvericin (BEA) using TLC and HPLC techniques. BEA was the highest frequency of mycotoxin detected, followed by MON, ZEN and FB_1. This study also presented the first report of BEA production by* F. solani, F. compactum *and* F. chlamydosporum. *All mycotoxins were not produced by* F. nelsonii and F. lateritium. *All* Fusarium *species were isolated from asymptomatic grasses, hence they are likely to exist as endophytes or latent pathogens.*

Keywords: Fusarium*; grasses; mycotoxins; Peninsular Malaysia*

ABSTRAK

Spesies Fusarium *dikenali sebagai penyebab pelbagai penyakit tumbuhan dan haiwan serta menghasilkan beberapa mikotoksin yang berbahaya. Penghasilan mikotoksin oleh spesies yang dipencilkan daripada perumah bukan pertanian seperti rumput liar, sehingga kini tidak pernah diberi perhatian. Kami memeriksa 30 strain mewakili 12 spesies* Fusarium *iaitu* F. oxysporum, F. solani, F. semitectum, F. nelsonii, F. compactum, F. equiseti, F. chlamydosporum, F. proliferatum, F. subglutinans, F. sacchari, F. lateritium *dan* F. incarnatum-equiseti *kompleks spesies yang dipencilkan daripada rumput liar di Semenanjung Malaysia untuk penghasilan empat mikotoksin utama iaitu moniliformin (MON), fumonisin B_1 (FB_1), zearalenon (ZEN) dan beauvericin (BEA) menggunakan teknik kromatografi lapisan nipis (TLC) dan kromatografi cecair berprestasi tinggi (HPLC). BEA merupakan mikotoksin yang paling kerap dikesan, diikuti oleh MON, ZEN dan FB_1. Kajian ini juga merupakan laporan pertama penghasilan BEA oleh* F. solani, F. compactum *dan* F. chlamydosporum. *Kesemua mikotoksin tidak dihasilkan oleh* F. nelsonii *dan* F. lateritium. *Semua spesies* Fusarium *tersebut dipencilkan daripada rumput yang tidak menunjukkan gejala penyakit, maka ia mungkin wujud sebagai endofit atau patogen pendam.*

Kata kunci: Fusarium*; mikotoksin*; *rumput; Semenanjung Malaysia*

INTRODUCTION

Several species of the genus *Fusarium* are known for causing serious plant diseases on a number of economically important plants worldwide, including those in Malaysia such as corn (Darnetty et al. 2008), rice (Nur Ain Izzati et al. 2008), sugar cane (Siti Nordahliawate et al. 2008) and banana (Liew et al. 1998). Members of this genus also produce harmful secondary metabolites known as mycotoxins (Desjardins 2006) in food and feeds. The four most important mycotoxins produced by *Fusarium* are zearalenone (ZEN), moniliformin (MON), fumonisin B_1 (FB_1) and beauvericin (BEA) (Leslie et al. 2004; Logrieco et al. 2002; Sopterean & Puia 2012). The possible health risks on animals and humans have evoked global concern over food safety and therefore, numerous research works have been focused on the toxicology of *Fusarium* mycotoxins (Lee et al. 2010; Negedu et al. 2011; Tan et al. 2012). ZEN has always been postulated to cause infertility among mammals, mammary hypertrophy in females and feminisation in males (Dacasto et al. 1995; Smith et al. 1994) with swine being the most vulnerable animal towards this toxin. Several studies showed MON is toxic, causing muscular weakness, respiratory distress, coma and even lead to fatality in tested animals (Engelhardt et al. 1989; Ledoux et al. 1995). The most recent study by Jonsson et al. (2012) and Sharma et al. (2012) have confirmed that heart is the main target tissue of MON toxicity in rats and avian. Among series of fumonisins, FB_1 is the most prominent and frequently found in foods and feeds. Over the years, FB_1 has been linked to leukoencephalomalacia, a brain lesion in horses (Marasas et al. 1988), human oesophageal cancer (Sydenham et al. 1990), immunodepressive effects in turkey poults (Li et al. 2000) and several others diseases. Apart from the above mentioned mycotoxins, BEA is regarded as a new and less investigated secondary metabolites since its role as a toxin is poorly understood (Jestoi 2008). However, some studies showed that BEA has an insecticidal activity (Gupta et al. 1991), induces programmed cell death similar to apoptosis (Macchia et al. 2002) and causes chronotropic effect, a decrease in the frequency of cardiac spontaneous beating activity in guinea pig heart (Lemmens-Gruber et al. 2000).

In Malaysia, very limited number of studies on mycotoxin have been conducted. Desjardins et al. (1997) performed a study of FB_1 and MON production in rice infected by *G. fujikuroi* and the results showed the rice samples were contaminated with FB_1 and MON at concentration levels of 170 µg/g and 1000 – 5000 µg/g, respectively. Other studies were carried out by several researchers mostly on edible products e.g. on Malay traditional vegetables (Nur Ain Izzati & Wan Hasmida 2011), corns and animal feeds (Reddy & Salleh 2011) and cereals (Soleimany et al. 2011). All results showed certain amounts of mycotoxins contamination in tested samples. To date, there is no scientifically reliable data regarding mycotoxins production by *Fusarium* species from non-agricultural hosts, particularly wild grasses. Like any other plants, grasses are also suitable hosts for *Fusarium* species. Leslie et al. (2004) initiated a study on species diversity and mycotoxins production by *Gibberella fujikuroi* (*Fusarium* section Liseola) recovered from prairie grasses in Kansas and found generally low to moderate amounts of fumonisins (120 µg/g), BEA (4 – 320 µg/g) and fusaproliferin (50 – 540 µg/g). Nur Ain Izzati et al. (2009) have isolated and reported 10 *Fusarium* species i.e. *F. semitectum, F. solani, F. oxysporum, F. equiseti, F. sacchari, F. proliferatum, F. subglutinans, F. compactum, F. longipes* and *F. chlamydosporum* from 25 samples of wild grasses in Peninsular Malaysia; indicating the possibility for multiple mycotoxins production by these species *in planta*. Therefore, a well-defined attention should be given to the incidence, type and chemical natures of *Fusarium* toxins in this area since in Malaysia, wild grasses are commonly used as feeding stocks for ruminants which lead to health and safety issues.

Thus, the objective of this study was to examine the production of the four most common mycotoxins produced by *Fusarium* species isolated from wild grasses in Peninsular Malaysia, both qualitatively and quantitatively, with the aim of providing an additional data on mycotoxins occurrence in Malaysian samples.

Materials and Methods

FUSARIUM STRAINS

Thirty strains of *Fusarium* isolated from 18 species of asymptomatic grasses (Table 1) collected throughout Peninsular Malaysia were examined for mycotoxins production *in vitro*. Strains were identified into species level following Leslie and Summerell (2006) as well as molecular approach described by O'Donnell et al. (1998), respectively. Strains were preserved in 15% glycerol (Salleh & Sulaiman 1984), catalogued and deposited at the Fusarium Culture Collection Unit, Universiti Sains Malaysia.

STANDARD SOLUTION OF MYCOTOXINS

All four mycotoxin standards were purchased from Sigma-Aldrich (St. Louis, MO, USA), diluted in methanol and prepared at concentrations ranging from 5 to 25 µg/g. The standard solutions were kept at 4°C for longer shelf life.

MYCOTOXINS PRODUCTION *IN VITRO*

Eighty five grams of corn grits (~ 45% moisture) were autoclaved in 250 mL Erlenmeyer flasks and inoculated with conidial suspensions (~1×10^7 conidia/mL) of *Fusarium* strains grown on potato dextrose agar (PDA) for 7 days. Corn grits inoculated with sterilised distilled water served as controls. The corn grit cultures and controls were incubated at 25 ± 2ºC for 28 days in total darkness.

MYCOTOXINS ANALYSES

Mycotoxins profiles of the 12 species of *Fusarium* strains were analyzed by using two techniques i.e. thin layer chromatography (TLC) for MON and FB_1 and high performance liquid chromatography (HPLC) for ZEN and BEA.

MONILIFORMIN (MON) AND FUMONISIN B_1 (FB_1)

The procedures of Burmeister et al. (1979) and Nelson et al. (1992) were adopted for extraction of MON and FB_1, respectively, with slight modifications. As for detection, plates were developed in a solvent system according to Thrane (1986) for MON and Nelson et al. (1992) for FB_1 and visualised under both white and UV (364 nm) lights. Retention factor (R_f) values for the standards and samples were calculated and compared.

ZEARALENONE (ZEN) AND BEAUVERICIN (BEA)

ZEN was extracted and analysed by techniques previously described by Bottalico et al. (1985) and Jimenez et al. (1997), with slight modifications. Meanwhile, BEA was extracted and analysed following the procedure of Logrieco et al. (1998) and Munkvold et al. (1998). The presence of

ZEN and BEA were confirmed and quantified by HPLC with a UV detector at 236 nm for ZEN and 205 nm for BEA at a flow rate of 0.6 mL/min. The extracts were injected into HPLC system and identified by comparing retention times and UV spectra of the samples with those of the standards and further quantified by comparing peak areas from the samples with a calibration curve of the standards.

Results and Discussion

We examined 30 strains of selected *Fusarium* species recovered from 18 species of wild grasses in producing four major mycotoxins i.e. MON, FB_1, ZEN and BEA. Out of 30 strains of 12 *Fusarium* species recovered, nine strains of seven species produced detectable levels of MON i.e. *F. oxysporum* (T3507&N, M3548&N, P3610&N), *F. chlamydosporum* (P3590&N), *F. solani* (J3517&N), *F. proliferatum* (D3474&N), *F. subglutinans* (T3503&N), *F. sacchari* (T3671&N) and *F. incarnatum-equiseti* species complex (C3485&N) (Table 1). The extracts of the tested strains showed the same R_f value (~0.68) and colour (bluish) as MON standard and are in agreement with previous reports (Mubatanhema et al. 1999; Thrane 1986). All species have been reported as MON producer (Chelkowski et al. 1990; Lew et al. 1996). One strain represented *F. incarnatum-equiseti* species complex was able to produce MON. Further work is therefore required to confirm the ability of this species to produce this toxin as no previous data was reported on the production of MON by *F. incarnatum-equiseti* species complex. Meanwhile, MON was absent in cultures inoculated with five *Fusarium* species i.e. *F. semitectum, F. equiseti*, *F. compactum*, *F. nelsonii* and *F. lateritium*. Similar result was obtained by Jimenez et al. (1997) as they did not detect any trace levels of MON by *F. semitectum* in their study and claimed that this species was a low MON producer. Nur Ain Izzati and Wan Hasmida (2011) however managed to detect MON in corn grit cultures inoculated with *F. semitectum* isolated from traditional vegetables in Malaysia. There is no credible explanation has been made to clarify as to why some strains within the species are able to produce MON and some are not. Meanwhile, the four latter species are considered as non- producer of MON (Leslie & Summerell 2006).

FB_1 was only detected in one strain of *F. proliferatum* (strain D3474&N) and in agreement with previous study by Logrieco et al. (2002). The R_f value for FB_1 was 0.22 and the colour appeared as light purple under the white light and reddish spot under the long wave UV (364 nm). The fact that no FB_1 was detected in two *Fusarium* species in section Liseola i.e. *F. subglutinans* and *F. sacchari* was also a common phenomenon as both species have been reported to produce this mycotoxin at very low or undetectable levels in corn grit cultures (Leslie et al. 1996; Reynoso et al. 2004; Tseng et al. 1995).

ZEN was only produced in corn grits inoculated with two species i.e. *F. semitectum* (C3482&N) and *F. equiseti* (M3543&N). *F. semitectum* and *F. equiseti* have been consistently classified as the main producers of ZEN (Frisvad & Thrane 2002; Hestbjerg et al. 2002). The concentration levels of ZEN produced by both species were low i.e. 2.8 and 4.4 μg/g, respectively and supported the proclamation by Kosiak et al. (2005) that countries with hot climate have not been presented with problems by ZEN contamination. Most strains of *F. oxysporum* did not produce ZEN; notwithstanding some reports accounted few strains of this species could produce ZEN (Marasas et al. 1984). The other 10 remaining species were reported as non-producers of ZEN (Desjardins 2006).

Nine species were able to produce detectable levels of BEA with low to moderate concentrations ranging from 19.5 to 567 μg/g. Two strains of *F. semitectum* (J3526&N and P3564&N), *F. equiseti* (D3741&N and C3494&N), *F. oxysporum* (T3507&N and P3610&N) and *F. proliferatum* (P3594&N and D3474&N) were positively detected for BEA and have been constantly reported as BEA producers (Leslie et al. 2004; Moretti et al. 2002). Meanwhile, only one strain each from *F. subglutinans* (T3503&N), *F. solani* (P3602&N), *F. compactum* (T3681&N) and *F. chlamydosporum* (D3696&N) produced BEA in the corn grit cultures. No earlier reports have been presented on the occurrence of BEA by *F. solani*, *F. compactum* and *F. chlamydosporum* (Leslie & Summerell 2006), as well as *F. incarnatum-equiseti* species complex. Hence, this study presented the first report of BEA production by these species. Several authors revealed that *F. subglutinans* was able to produce BEA in cultures (Leslie et al. 2004; Reynoso et al. 2004; Shephard et al. 1999) and in contrast with Moretti et al. (1996) and Munkvold et al. (1998) who found none BEA in *F. subglutinans* strains from corn samples in Iowa, Argentina and Italy. All strains of *F. sacchari* did not produce detectable levels of BEA. All four mycotoxins were apparently not produced by *F. nelsonii* and *F. lateritium*. Presumably the toxin profiles for this species is similar to those of the most closely related species from section Arthrosporiella i.e. *F. semitectum*. Meanwhile, *F. lateritium* was reported to produce other mycotoxins such as enniatins (Pieper et al. 1992) and lateropyrone (Bushnell et al. 1984).

Conclusion

Fusarium species isolated from wild grasses in Peninsular Malaysia were also able to produce the four major mycotoxins i.e. moniliformin (MON), fumonisin B_1 (FB_1), zearalenone (ZEN) and beauvericin (BEA). The results of this study may indicate a potential health risk for ruminants that feed on these grasses and consequently for humans who consume these animals as a protein source.

Acknowledgments

This research was supported by funding from the Research University Grant (FRGS, PBIOLOGI/6711157), Ministry of Science, Technology and Innovation (MOSTI). We thank Mr. Kamarudin Mohd Maidin for technical assistance.

TABLE 1. Production of four types of mycotoxins by 30 *Fusarium* strains isolated non-cultivated grasses in Peninsular Malaysia

Fusarium species[a]	Host[b]	Strains[c]	Location[d]	Mycotoxins (µg/g)			
F. semitectum	*Chloris barbata*	C3482&N	Temerloh, Pahang	n.d	n.d	+/4.4	n.d
	Paspalum conjugatum	J3526&N	Skudai, Johor	n.d	n.d	n.d	+/45
	Sporobolus diander	P3564&N	Seberang Perai, P. Pinang	n.d	n.d	n.d	+/25.5
F. solani	*Digitaria setigera*	P3602&N	Teluk Bahang, P. Pinang	n.d	n.d	n.d	+/39
	Axonopus compressus	J3517&N	Skudai, Johor	+/NA	n.d	n.d	n.d
	Digitaria ciliaris	D3477&N	Pasir Putih, Kelantan	n.d	n.d	n.d	n.d
F. equiseti	*Sporobolus diander*	D3741&N	Kubang Kerian, Kelantan	n.d	n.d	n.d	+/547.5
	Eleusine indica	M3543&N	Saint John, Melaka	n.d	n.d	+/2.8	n.d
	Paspalum conjugatum	C3494&N	Cameron Highlands, Pahang	n.d	n.d	n.d	+/36
F. compactum	*Imperata cylindrica*	T3681&N	Kemaman, Terengganu	n.d	n.d	n.d	+/18
	Cyanodon dactylon	K3638&N	Padang Terap, Kedah	n.d	n.d	n.d	n.d
	Axonopus compressus	J3523&N	Skudai, Johor	n.d	n.d	n.d	n.d
F. oxysporum	*Centhotheca lappacea*	T3507&N	Kemaman, Terengganu	+/NA	n.d	n.d	+/567
	Eragrostis amabilis	M3548&N	Saint John, Melaka	+/NA	n.d	n.d	n.d
	Lophatherum gracile	P3610&N	Teluk Bahang, P. Pinang	+/NA	n.d	n.d	+/228
F. proliferatum	*Echinochloa cruss-galli*	P3594&N	Relau, P. Pinang	n.d	n.d	n.d	+/235.5
	Eleusine indica	M3542&N	Saint John, Melaka	n.d	n.d	n.d	n.d
	Panicum repens	D3474&N	Pasir Putih, Kelantan	+/NA	+/NA	n.d	+/13.5
F. subglutinans	*Paspalum conjugatum*	T3514&N	Kemaman, Terengganu	n.d	n.d	n.d	n.d
	Paspalum orbiculare	C3856&N	Cameron Highlands, Pahang	n.d	n.d	n.d	n.d
	Centhotheca lappacea	T3503&N	Kemaman, Terengganu	+/NA	n.d	n.d	+/147
F. sacchari	*Eleusine indica*	K3619&N	Padang Terap, Kedah	n.d	n.d	n.d	n.d
	Paspalum conjugatum	J3527&N	Skudai, Johor	n.d	n.d	n.d	n.d
	Chrysopogon aciculatus	T3671&N	Kemaman, Terengganu	+/NA	n.d	n.d	n.d
F. chlamydosporum	*Eleusine indica*	D3696&N	Kuala Krai, Kelantan	n.d	n.d	n.d	+/12
	Pennisetum purpureum	P3590&N	Relau, P. Pinang	+/NA	n.d	n.d	n.d
	Imperata cylindrica	K3634&N	Padang Terap, Kedah	n.d	n.d	n.d	n.d
F. nelsonii	*Echinochloa colona*	B3850&N	Sg. Besar, Selangor	n.d	n.d	n.d	n.d
F. lateritium	*Eragrostis amabilis*	M3550&N	Saint John, Melaka	n.d	n.d	n.d	n.d
F. incarnatum-equiseti species complex[e]	*Axonopus compressus*	C3485&N	Cameron Highlands, Pahang	+/NA	n.d	n.d	+/4.5

[a]*Fusarium* species identified according to Leslie and Summerell (2006); [b]Host of each *Fusarium* species isolated; [c] Strain, a coding system by *Fusarium* Collection Unit, Universiti Sains Malaysia, MALAYSIA; (initial alphabet = states in Malaysia; ampersand (&) = grasses/weeds, 'N' = non-pathogenic); [d]Sampling sites in Peninsular Malaysia; [e]Species identified based on gene sequencing analyse; + = presence; n.d = not detected; NA = concentration values not available

REFERENCES

Bottalico, A., Visconti, A., Logrieco, A., Solfrizzo, M. & Mirocha, J. 1985. Occurrence of zearalenols (diastereomeric mixture) in corn stalk rot and their production by associated *Fusarium* species. *Applied and Environmental Microbiology* 49: 547-551.

Burmeister, H.R., Ciegler, A. & Vesonder, R.F. 1979. Moniliformin, a metabolite of *Fusarium moniliforme* NRRL 6322 - purification and toxicity. *Applied and Environmental Microbiology* 37: 11-13.

Bushnell, G.W., Li, Y.L. & Poulton, G.A. 1984. Pyrones. X. Lateropyrone, a new antibiotic from the fungus *Fusarium lateritium* Nees. *Canadian Journal of Chemistry* 62: 2101-2106.

Chelkowski, J., Zawadzki, M., Zajkowski, P., Logrieco, A. & Bottalico, A. 1990. Moniliformin production by *Fusarium* species. *Mycotoxin Research* 6: 41-45.

Dacasto, M., Rolando, P., Nachtmann, C., Ceppa, L. & Nebbia, C. 1995. Zearalenone mycotoxicosis in piglets suckling sows fed contaminated grain. *Veterinary and Human Toxicology* 37: 359-361.

Darnetty., Dian Mariana, M.N., Nur Ain Izzati, M.Z., Nor Azliza, I., Nik Mohd Izham, N.M. & Salleh, B. 2008. Diversity of *Fusarium* species associated with ear rot of corn in Indonesia and Malaysia. In *Proceedings of 6th IMT-GT Conference, Penang*, 26-28 August, edited by Abd Wahab, A.R., Chan, L.K., Shaida Fariza, S., Ahmad Ramli, M.Y., Amirul Al-Ashraf, B., Zairi, J., Rashidah, A.R., Sudesh, K., Siti Azizah, M.N., Darah, L., Zary Shariman, Y., Aileen, T.S.H. & Alexander, C.

Desjardins, A.E. 2006. *Fusarium Mycotoxins: Chemistry, Genetics, and Biology*. St. Paul, Minnesota: Amer Phytopathological Society.

Desjardins, A.E., Plattner, R.D. & Nelson, P.E. 1997. Production of fumonisin B_1 and moniliformin by *Gibberella fujikuroi* from rice from various geographic areas. *Applied and Environmental Microbiology* 63: 1838-1842.

Engelhardt, J.A., Carlton, W.W. & Tuite, J.F. 1989. Toxicity of *Fusarium moniliforme* var. s*ubglutinans* for chicks, ducklings, and turkey poults. *Avian Diseases* 33: 357-360.

Frisvad, J.C. & Thrane, U. 2002. Mycotoxin production by common filamentous fungi. In *Introduction to Food- and Air-borne Fungi,* edited by Samson, R.A., Hoekstra, E.S., Frisvad, J.C., Filtenborg, O. Utrecht: Centraalbereau voor Schimmelcultures. pp. 321-331.

Gupta, S., Krasnoff, B., Underwood, N.L., Renwick, J.A.A. & Roberts, D.W. 1991. Isolation of beauvericin as an insect toxin from *Fusarium semitectum* and *Fusarium moniliforme* var. *subglutinans*. *Mycopathologia* 115: 185-189.

Hestbjerg, H., Nielsen, K.F., Thrane, U. & Elmholt, S. 2002. Production of trichothecenes and other secondary metabolites by *Fusarium culmorum* and *Fusarium equiseti* on common laboratory media and a soil organic matter agar: An ecological interpretation. *Journal of Agricultural and Food Chemistry* 50: 7593 -7599.

Jestoi, M. 2008. Emerging *Fusarium*-mycotoxins fusaproliferin, beauvericin, enniatins, and moniliformin: A review. *Critical Reviews in Food Science and Nutrition* 48: 21-49.

Jimenez, M., Huerta, T. & Mateo, R. 1997. Mycotoxin production by *Fusarium* species isolated from bananas. *Applied and Environmental Microbiology* 63: 364-369.

Jonsson, M., Jestoi, M., Nathanail, A.V., Kokkonen, U-M., Koivisto, P., Karhunen, P. & Peltonen, K. 2012. Application of OECD Guideline 423 in assessing the acute oral toxicity of moniliformin. *Food and Chemical Toxicology* 53: 27-32.

Kosiak, E.B., Holst-Jensen, A., Rundburget, T., Jaen, M.T.G. & Torp, M. 2005. Morphological, chemical and molecular differentiation of *Fusarium equiseti* isolated from Norwegian cereals. *International Journal of Food Microbiology* 99: 195-206.

Lee, K-E., Kim, B.H. & Lee, C. 2010. Occurrence of *Fusarium* mycotoxin beauvericin in animal feeds in Korea. *Animal Feed Science and Technology* 157: 190-194.

Lemmens-Gruber, R., Rachoy, B., Steininger, E., Kouri, K., Saleh, P., Krska, R., Josephs, R. & Lemmens, M. 2000. The effect of the *Fusarium* metabolite beauvericin on electromechanical and physiological properties in isolated smooth and heart muscle preparations of guinea pigs. *Mycopathologia* 49: 5-12.

Leslie, J.F. & Summerell, B.A. 2006. *The Fusarium Laboratory Manual*. UK: Blackwell Publishing Ltd.

Leslie, J.F., Marasas, W.F.O., Shephard, G.S., Sydenham, E.W., Stockenstrom, S. & Thiel, P.G. 1996. Duckling toxicity and the production of fumonisin and moniliformin by isolates in the A and F mating populations of *Gibberella fujikuroi* (*Fusarium moniliforme*). *Applied and Environmental Microbiology* 62: 1182-1187.

Leslie, J.F., Zeller, K.A., Logrieco, A., Mulè, G., Moretti, A. & Ritieni, A. 2004. Species diversity and toxin production by strains in the *Gibberella fujikuroi* species complex isolated from native prairie grasses in Kansas. *Applied and Environmental Microbiology* 70: 2254-2262.

Ledoux, D.R., Bermudez, A.J., Rottinghaus, G.E., Broomhead, J. & Bennett, G.A. 1995. Effects of feeding *Fusarium fujikuroi* culture material, containing known levels of moniliformin, in young broiler chicks. *Poultry Science* 74: 297-305.

Lew, H., Chelkowski, J., Pronczuk, P. & Edinger, W. 1996. Occurrence of the mycotoxin moniliformin in maize (*Zea mays* L.) ears infected by *Fusarium subglutinans* (Wollenw. & Reinking). *Food Additives and Contaminants* 13: 321-324.

Li, Y.C., Ledoux, D.R., Bermudez, A.J., Fritsche, K.L. & Rottinghaus, G.E. 2000. The individual and combined effects of fumonisin B_1 and moniliformin on performance and selected immune parameters in turkey poults. *Poultry Science* 79: 871-878.

Liew, K.W., Mak, C. & Ho, Y.W. 1998. Status of banana Fusarium wilt in Malaysia. In *Proceedings of the First Banana Seminar, 23 – 25 November,* edited by Zakaria, W., Mahmud, T.M.M., Siti, K.D., Nor 'Aini, M.F. & Marziah, M.

Logrieco, A., Moretti, A., Castellá, G., Kostecki, M., Golinski, P., Ritieni, A. & Chelkowski, J. 1998. Beauvericin production by *Fusarium* species. *Applied and Environmental Microbiology* 64: 3084-3088.

Logrieco, A., Rizzo, A., Ferracane, R. & Ritieni, A. 2002. Occurrence of beauvericin and enniatins in wheat affected by *Fusarium avenaceum* head blight. *Applied and Environmental Microbiology* 68: 82-85.

Macchia, L., Caiffa, M.F., Fornelli, F., Calo, L., Nenna, S., Moretti, A., Logrieco, A. & Tursi, A. 2002. Apoptosis induced by the *Fusarium* mycotoxin beauvericin in mammalian cells. *Journal of Applied Genetics* 43: 363-371.

Marasas, W.F.O., Nelson, P.E. & Toussoun, T.A. 1984. *Toxigenic Fusarium Species: Identity and Mycotoxicology*. University Park, Pennsylvania: The Pennsylvania State University Press.

Marasas, W.F.O., Kellerman, T.S. & Gelderbom, W.C.A. 1988. Leukoencephalomalacia in a horse induced by fumonisin B_1

isolated from *Fusarium moniliforme*. *Onderstepoort Journal of Veterinary Research* 43: 113-122.

Moretti, A., Belisario, A., Tafuri, A., Ritieni, A., Corazza, L. & Logrieco, A. 2002. Production of beauvericin by different races of *Fusarium oxysporum* f. sp. *melonis*, the Fusarium wilt agent of muskmelon. *European Journal of Plant Pathology* 108: 661-666.

Moretti, A., Logrieco, A., Bottalico, A., Ritieni, A., Fogliano, V. & Randazzo, G. 1996. Diversity in beauvericin and fusaproliferin production by different populations of *Gibberella fujikuroi* (*Fusarium* section *Liseola*). *Sydowia* 30: 44-56.

Mubatanhema, W., Moss, M.O., Frank, M.J. & Wilson, D.M. 1999. Prevalence of *Fusarium* species of the Liseola section on Zimbabwean corn and their ability to produce the mycotoxins zearalenone, moniliformin and fumonisin B_1. *Mycopathologia* 148: 157-163.

Munkvold, G., Stahr, H.M., Logrieco, A., Moretti, A. & Ritieni, A. 1998. Occurrence of fusaproliferin and beauvericin in *Fusarium*-contaminated livestock feed in Iowa. *Applied and Environmental Microbiology* 64: 3923-3926.

Negedu, A., Atawodi, S.E., Ameh, J.B., Umoh, V.J. & Tanko, H.Y. 2011. Economic and health perspectives of mycotoxins: A review. *Continental Journal of Biomedical Sciences* 5: 5-26.

Nelson, P.E., Plattner, R.D., Shackelford, D.D. & Desjardins, A.E. 1992. Fumonisin B_1 production by *Fusarium* species other than *F. moniliforme* in section *Liseola* and some related species. *Applied and Environmental Microbiology* 58: 984-989.

Nur Ain Izzati, M.Z. & Wan Hasmida, W.I. 2011. Isolation of microfungi from traditional vegetables and secondary metabolites produced by *Fusarium* species. *Sains Malaysiana* 40: 437-444.

Nur Ain Izzati, M.Z., Azmi, A.R. & Salleh, B. 2008. Bakanae disease of rice in Malaysia and Indonesia: Etiology of the causal agent based on morphological, physiological and pathogenicity characteristics. *Journal of Plant Protection Research* 48: 491-501.

Nur Ain Izzati, M.Z., Siti Nordahliawate, M.S., Nor Azliza, I. & Salleh, B. 2009. Distribution and diversity of *Fusarium* spp. associated with grasses in ten states throughout Peninsular Malaysia. *Biotropia* 16: 55-64.

O'Donnell, K., Kistler, H.C., Cigelnik, E. & Ploetz, R.C. 1998. Multiple evolutionary origins of the fungus causing Panama disease of banana: Concordant evidence from nuclear and mitochondrial gene genealogies. *Proceedings of the National Academy of Sciences of the United States of America* 95: 2044-2049.

Pieper, R., Kleinkauf, H. & Zocher, R. 1992. Enniatin synthetases from different Fusaria exhibiting distinct amino acid specificities. *Journal of Antibiotics* 45: 1273-1277.

Reddy, K.R.N. & Salleh, B. 2011. Co-occurrence of moulds and mycotoxins in corn grain used for animal feeds in Malaysia. *Journal of Veterinary Advances* 10: 668-673.

Reynoso, M.M., Torres, A.M. & Chulze, S.N. 2004. Fusaproliferin, beauvericin and fumonisin production by different mating populations among the *Gibberella fujikuroi* species complex isolated from maize. *Mycological Research* 108: 154-160.

Salleh, B. & Sulaiman, B. 1984. Fusaria associated with naturally diseased plants in Penang. *Journal of Plant Protection in the Tropics* 1: 47-53.

Sharma, D., Asrani, R.K., Ledoux, D.R., Rottinghaus, G.E. & Gupta, V.K. 2012. Toxic interaction between fumonisin B_1 and moniliformin for cardiac lesions in Japanese quail. *Avian Diseases* 56: 545-554.

Shephard, G.S., Sewram, V., Nieuwoudt, T.W., Marasas, W.F.O. & Ritieni, A. 1999. Production of the mycotoxins fusaproliferin and beauvericin by South African isolates in the *Fusarium* section *Liseola*. *Journal of Agriculture and Food Chemistry* 47: 5111-5115.

Siti Nordahliawate, M.S., Nur Ain Izzati, M.Z., Azmi, A.R. & Salleh, B. 2008. Distribution, morphological characteristics and pathogenicity of *Fusarium sacchari* associated with pokkah boeng disease on sugarcane in Peninsular Malaysia. *Pertanika Journal of Tropical Agricultural and Science* 31: 271-278.

Smith, J.E., Lewis, C.W., Anderson, J.G. & Solomons, G.L. 1994. *Mycotoxins in Human Nutrition and Health*. Directorate General XII. Science, Research and Development. EUR 16048 EN.

Soleimany, F., Jinap, S., Rahmani, A. & Khatib, A. 2011. Simultaneous detection of 12 mycotoxins in cereals using RP-HPLC-PDA-FLD with PHRED and a post-column derivatization system. *Food Additives and Contaminants* 28: 494-501.

Sopterean, L.M & Puia, C. 2012. The major mycotoxins produced by *Fusarium* fungi and their effects. *ProEnvironment* 5: 55-59.

Sydenham, E.W., Thiel, P.G., Marasas, W.F.O., Shephard, G.S., van Schalkwyk, D.J. & Koch, K.R. 1990. Natural occurrence of some *Fusarium* mycotoxins in corn from low and high oesophageal cancer prevalence areas of the Transkei, Southern Africa. *Journal of Agricultural and Food Chemistry* 38: 1900-1903.

Tan, D.C., Flematti, G.R., Ghisalberti, E.L., Sivasithamparam, K., Chakraborty, S., Obanor, F., Jayasena, K. & Barbetti, M.J. 2012. Mycotoxins produced by *Fusarium* spp. associated with Fusarium head blight of wheat in Western Australia. *Mycotoxin Research* 28: 89-96.

Thrane, U. 1986. Detection of toxigenic *Fusarium* isolates by thin layer chromatography. *Letters in Applied Microbiology* 3: 93-96.

Tseng, T.C., Tu, J.C. & Tzean, S.S. 1995. Mycoflora and mycotoxins in dry bean (*Phaseolus vulgaris*) produced in Taiwan and in Ontario, Canada. *Botanical Bulletin of Academia Sinica* 36: 229-234.

School of Biological Sciences
Universiti Sains Malaysia
11800 Minden, Penang
Malaysia

*Corresponding author; email: sallehb@usm.my

Received: 11 September 2012
Accepted: 19 April 2013